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(54) LIQUID MEMBRANE ENCAPSULATED MEDICINALS AND USES THEREOF

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ABSTRACT OF DISCLOSURE

A method for removing a toxin from the gastro-intestinal tract which comprises providing an emulsion in said gastro-intestinal tract which comprises an interior phase surrounded by a surfactant-containing exterior phase, said exterior phase being immiscible with the aqueous environment of said gastro-intestinal tract and permeable to said toxins, said emulsion being further characterized as being stable in said gastro-intestinal tract, and said interior phase comprising (a) a reactant capable of converting said toxin into a non-permeable form, whereby said toxin permeates the exterior phase of said emulsion into said interior phase and is converted into a non-permeable form or (b) a catalyst which is insoluble in said exterior phase and capable of converting said toxin, whereby said toxin permeates the exterior phase and is converted in said interior phase.

THE EMBODIMENTS OF THE INVENTION IN WHICH AN EXCLUSIVE

PROPERTY OR PRIVILEGE IS CLAIMED ARE DEFINED AS FOLLOWS:

1. An emulsion useful in removing or converting a toxin of the gastrointestinal tract which comprises an interior phase surrounded by an exterior phase, said exterior phase being immiscible with the aqueous environment of said gastrointestinal tract and permeable to said toxin, said exterior phase comprising an oil-soluble surfactant component and an oil component, said oil-soluble surfactant component and said oil component being harmless to the human body, said oil-soluble surfactant component being present in said exterior phase from about 0.01 wt. % to 90 wt. % of said exterior phase, said oil component having a viscosity between about 2 and about 1,000 centistokes at normal body temperature and selected from the group consisting of vegetable oils and animal fats that are heavily hydrogenated to contain at least 10% more hydrogen than normal saturation, perfluorinated hydrocarbons, silicone fluids containing the repeating unit

$$\begin{array}{c} CH_3 \\ | \\ -Si-O \\ | \\ CH_3 \end{array}$$

and hydrocarbon oils refined to remove toxic ingredients and comprising molecular weights up to 1,000, selected from the group consisting of paraffins, isoparaffins, naphthenes, and aromatics and said interior phase comprising a non-permeating reactant capable of converting said toxin into non-permeating form or a catalyst which is insoluble in said exterior phase and capable of converting said toxin into a non-toxin.

2. The emulsion of claim 1 wherein said oil-soluble surfactant component is present in said exterior phase from about 0.01 wt. % to 10 wt. % of said exterior phase.

3. The emulsion of claim 1 wherein said emulsion is suspended in a liquid which is not harmful to the human body and said liquid is immiscible with said exterior phase of said emulsion.

4. The emulsion of claim 1 wherein a strengthening agent is included in said emulsion to improve said emulsion's stability.

5. The emulsion of claim 1 wherein said toxin is ammonia.

6. The emulsion of claim 1 or 5 wherein said reactant is an acid.

7. The emulsion of Claim 1 wherein the aqueous interior phase comprises an enzyme catalyst.

8. The emulsion of Claim 7 wherein said interior phase comprises urease which is available to convert urea.

9. The emulsion of Claim 1 wherein said oil component is a hydrocarbon oil refined to remove toxic ingredients and comprises molecular weights up to 1,000, selected from the group consisting of paraffins, isoparaffins, naphthenes, and aromatics.

BACKGROUND OF THE PRIOR ART

FIELD OF THE INVENTION

This invention relates to the use of liquid membrane technology in preparing medicinals. The medicinals prepared by this invention may be ingested and may be utilized as traps for toxins present in the GI (gastrointestinal) tract, or as slow release compositions of drugs, or as reactors. In the trap embodiment the liquid membrane encapsulated medicinal is an emulsion comprising an external phase which is immiscible with the liquids present in the GI tract and permeable to the toxins therein, and an interior phase which comprises a reagent capable of converting said toxin to a nonpermeable form. In addition, hydrophilic adsorbents may be encapsulated such as a hydrophilic carbon or a silica gel. When the compositions of the instant invention are utilized as slow release drugs, the internal phase of the emulsion will comprise a drug which is slightly soluble in the external phase of the emulsion whereby said drug permeates through said exterior phase of the emulsion over a period of time into the GI tract. The third method for utilizing the compositions of the instant invention comprises encapsulating a catalyst for a reaction which is desired to be carried out in the GI tract. In this embodiment the reactants present in the GI tract permeate through the external phase of the emulsion into an interior phase wherein said catalyst, for example, an enzyme is immobilized and are converted to reaction products which then may permeate through the external phase back into the GI tract. In all cases the liquid membrane encapsulated medicinals may be administered by either oral ingestion or injection anywhere else into the GI tract.

SUMMARY OF THE PRIOR ART

It is known in the art that solid microcapsules may be utilized to encapsulate medicinals. For example, in the December 20, 1971 issue of "The Journal of the American Medical Association" in the "Medical News Section", a review of the microencapsulated medicinal art is presented. In this article, a technique for treating uremic wastes in the gastrointestinal tract with microencapsulated activated carbon is disclosed. The microcapsule is permeable to the uremic wastes and said activated carbon is utilized to absorb some of the wastes. In this technique, uric acid and creatinine are removed. The above reference also teaches a technique wherein urea is converted to ammonia and CO_2 by the use of microencapsulated urease. The ammonia is then reacted with and trapped by a microencapsulated ethylene maleic acid copolymer while the carbon dioxide is exhaled through the lungs.

It is known in the art of slow release medicinals that medicinals can be encapsulated by various solid materials, for example, hydroxy alkyl cellulose ethers, as taught in U.S. Patent 3,493,407, and gelatin, as taught in U.S. Patent 3,526,682. In both of these patents, the microencapsulated medicinal is released over a time period into the GI tract by dissolution of the solid capsule material.

There are various problems known in the art in using solid microcapsules as reactors, as traps and as slow release compositions. One problem is that solid capsules are prone to swell followed by rupture and indeed various methods to solve this problem have been utilized, including crenation, etc. This process increases the cost of microencapsulated systems and when long residence times in

the GI tract are encountered, these crenated compounds or compositions still rupture to an undesirable extent. Furthermore, the microencapsules which do not dissolve in the tract often lead to fecal compaction. In the compositions of the instant invention, the encapsulating medium is liquid; thus, expansion of the internal phase does not lead to ruptue of the composition as in the solid microencapsulated system disclosed above.

As pointed out in the patents cited above, when gelatin is utilized to encapsulate medicinals to provide slow release, various conditions encountered during storage can affect the rate of release in the GI tract. For example, gelatin is very sensitive to temperature and humidity, etc. In the emulsion systems of the instant invention, storage conditions do not substantially affect the rate of release of the compositions in the GI tract.

U.S. 3,538,216 describes an invention in which a thixotropic or gelatinous oil containing a drug for sustained release is injected into an animal. The instant invention is quite different in that a suspendable emulsion is ingested.

SUMMARY OF THE INVENTION

The instant invention relates to medicinal compounds which comprise a medicinal emulsified in a water immiscible external phase. The emulsion is designed to be stable during passage through the GI tract where said medicinals will be utilized. To prepare the composition of the instant invention, the medicinal is usually dissolved in an aqueous medium and the solution thereof emulsified in an oil which is immiscible with the liquids present in the GI tract. The oil phase would generally contain a surfactant to enable the preparation of an

emulsion which will be stable during passage through the GI tract. The external phase of the emulsion thus acts like a liquid membrane surrounding the internal phase.

In a preferred embodiment, the emulsion described above is further dispersed in a liquid which is immiscible with the exterior phase of the emulsion, for example, water. This preferred embodiment allows the use of the compositions of the instant invention in a form wherein dispersion of the emulsion in the GI tract is increased. Furthermore, because of the well known unpalatability of the usual oils which are used to form the emulsions of the instant invention, the continuous phase comprising water or water and flavoring agents is desirable.

The compositions of the instant invention can be utilized in three different manners. For example, to remove toxins, reactants and adsorbents can be emulsified in the interior phase of an emulsion. The exterior phase of this emulsion will be designed to allow the toxins present in the GI tract to permeate through and react with the reactant or be adsorbed on the adsorbent present in the internal phase of the emulsion. In this manner, toxins are continuously and irreversibly removed as the emulsion passes through the GI tract. In this technique, the membrane is designed to be impermeable to the reaction products or adsorbed products formed in the interior phase of the emulsion.

In an alternate method, the liquid membrane is utilized to encapsulate catalysts which will be used in carrying out reactions while passing through the GI tract. The catalyst may be, for example, an enzyme, e.g. urease. Because of the liquid membrane encapsulating the catalysts, the catalyst itself can be used under conditions where the

catalyst in an uncapsulated state would be destroyed. For example, urease could be protected from the low pH present in the stomach of the GI tract by designing the liquid membrane to exclude the passage of ions including hydrogen ion.

In the third use of the compositions of the instant invention, medicinals are released by permeating through the exterior phase of the emulsion into the GI tract during the passage of the emulsion through the GI tract. In this embodiment, the medicinal compound is emulsified in a liquid in which the medicinal is only sparingly soluble. This low solubility in the external phase of the emulsion allows passage of the medicinal into the GI tract over long time periods.

In preparing the compositions of the instant invention, it is desirable to incorporate a surfactant in the external phase. This surfactant may be present in amounts from .01 to 90% wt. of said external phase. Preferably, the surfactant will be present in amounts from 1 to 5 wt. % of said external phase. The external phase is generally made up of the surfactant and an oil. The oil, of course, is designed to be immiscible with the liquids present in the GI tract. A further qualification for oils which may be utilized in preparing the compositions of the instant invention is that the oils must not be harmful to the human body. These oils along with the surfactant should also be fairly inert so that they are not destroyed by the environment in the GI tract.

The body digests many of the natural animal and vegetable oils. These readily digested oils such as triglycerides cannot be used to form a large fraction of the oil in the external phase. The natural digestive processes

would be expected to remove these oils from the emulsion as it passed through the GI tract.

It is well known in the art that "most artificial or natural emulsions are broken in the stomach". See, for example, Physiology of the Digestive Tract, H. W. Davenport, 3rd Ed. 1971 Year Book Medical Publishers, Inc., Chicago, Ill., page 197. It requires a special type of emulsion composition to pass through the GI tract intact. Some examples of oils which can be utilized in forming the compositions of the instant invention include hydrocarbon oils, e.g. paraffins, isoparaffins, naphthenes, and aromatics, having molecular weights up to 1,000. Particularly desirable are the mineral oils which have been highly refined for use in human ingestion. Additionally, oils or treated oils from animal or vegetable sources may be used if they can pass through the GI tract substantially unconverted, for example, vegetable oils and animal fats that are heavily hydrogenated so as to contain at least 10 wt. % more hydrogen than at normal saturation. Further, silicone fluids containing the repeating unit $\begin{matrix} CH_3 \\ | \\ -Si-O- \\ | \\ CH_3 \end{matrix}$ can be used. Perfluorinated hydrocarbons may also be used. Any of these oils should have a viscosity of 2 to 1000 centistokes at normal body temperature. The preferable range is 10 to 150 centistokes.

The surfactants must also be harmless to the human body if they are to be utilized in the instant invention. The specific surfactants which can be used in preparing the emulsions above include sorbitan monooleate and other types of sorbitan fatty acid esters, e.g. sorbitan, sorbitan monolaurate, sorbitan monopalmitate, sorbitan

stearate, sorbitan tristearate, sorbitan trioleate; polyoxyethylene sorbitan fatty acid esters; and mono- and diglycerides.

It may also be desirable to use strengthening agents to improve the stability of the emulsions. Nonlimiting examples of strengthening agents include; polyisobutylene, i.e. especially the lower molecular weights, e.g. a molecular weight of about 900, polyisobutylene succinic anhydride-pentaerythritol adducts, ethylene-vinyl acetate copolymers, sulfonated butyl rubber and decylmethacrylate-vinyl pyridine copolymers.

EXAMPLE 1

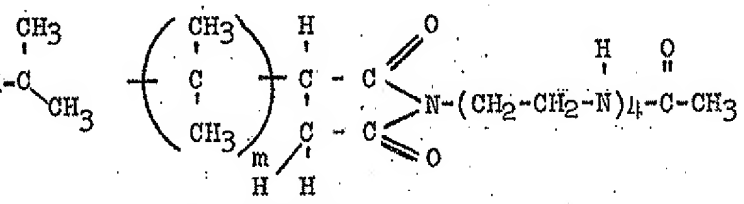
A controlled release medicinal (sodium salicylate.)

In experiment 1, a solution of 8 wt. % sodium salicylate and 8 wt. % sucrose in distilled water was used to form the internal phase of the emulsion. Experiment 2 used 10 wt. % sodium salicylate and 8 wt. % sucrose in water, but was the same as experiment 1 in all other respects.

These internal phases were added with vigorous agitation, in an amount sufficient to form 33 wt. % of the final emulsion, to an oil phase consisting of:

2.0 wt. % Sorbitan monooleate

0.5 wt. % of a high molecular weight polyamine with the structure:



wherein m is an integer of about 40

3.5 wt. % of a polyisobutylene with a molecular weight of about 900

94.0 wt. % of an isoparaffinic lubricating oil stock with a viscosity at 100°F of about 100 Saybolt Universal seconds.

200 grams of this emulsion was suspended in 600 grams of a synthetic gut fluid which comprised:

0.8 wt. % albumin from eggs

0.5 wt. % NaCl

0.4 wt. % $NaHCO_3$

98.3 wt. % distilled water

with mild agitation to simulate conditions in the small intestine. The appearance of sodium salicylate and sucrose in the bulk synthetic gut fluid were monitored with time by analysis. The results are shown in Table 1 below

Table 1

Controlled Release of Medicinals (Sodium Salicylate) by Diffusion in Liquid Membrane

	Concn. in External Phase, % (1)		% of Max. Equilibrium Concn. in Outer Phase	
Time Hrs.	Sodium Salicylate	Sucrose	Sodium Salicylate	Sucrose
Expt. #1				
0	0.0	0.005	0.0	0.5
80	0.83	0.010	64.0	1.0
Expt. #2				
0	0.0	0.008	0.0	1.0
80	0.59	0.013	45.0	1.6

As can be seen from the above table, the sucrose was at least 98 percent contained over the 80 hour period in both the experiments which indicates that the emulsions remained substantially intact. The controlled release of sodium salicylate was demonstrated by releasing 64 and 45 percent respectively of the maximum possible amounts over the 80 hour period.

The selection of the internal phase of the emulsions of the instant invention is dependent on their intended use. For example, toxins present in the GI tract may be removed by trapping them in the internal phase of the emulsion, i.e. conversion of a toxin which can permeate the external phase of the emulsion, to an impermeable form. Toxins may also be converted, in the internal phase, to an innocuous form, or alternatively to a form which may be subsequently trapped. An example of this technique is the conversion of urea, by use of urease, into carbon dioxide,

which may be exhaled, and ammonia, which may be trapped by an encapsulated strong acid.

The various toxins which may be removed from the GI tract by trapping in the internal phase of the compositions of the instant invention include

Table 2

Toxin Removal with Reagents Encapsulated in Liquid Membranes

Toxin	Reagents
Ammonia	Acid - preferably hydrochloric, sulfuric or citric
Phenol	Base - preferably sodium hydroxide
Phosphate	Calcium Salts - preferably a combination of calcium chloride and calcium hydroxide
Lactic Acid	Base - preferably sodium hydroxide
Iron	Base - preferably sodium hydroxide
Copper	Sulfide - preferably sodium sulfide
Silver	Sulfide - preferably sodium sulfide
Mercury	Sulfide - preferably sodium sulfide

Examples of using the instant invention to convert materials present in the GI tract into useful products include:

(1) The use of liquid membrane encapsulated amylase (an enzyme for the hydrolysis of starches for the digestion of starches),

(2) The use of liquid membrane encapsulated lipase (an enzyme for the hydrolysis of triglycerides for the digestion of triglycerides),

(3) The use of liquid membrane encapsulated lactase to help young children hydrolyze lactose,

(4) The use of liquid membrane encapsulated mixed pancreatic enzymes to promote the digestion and utilization of proteins in children with cystic fibrosis.

Other examples of using the compositions of the instant invention as reactors, wherein materials which are not capable of being trapped by reaction or adsorption in the internal phase of emulsions of the instant invention are converted into products which can be so trapped or adsorbed, include converting glucose to gluconic acid, and, lactose to lactic acid.

The compositions of the instant invention may be utilized as slow release medicinals to release, for example, Sodium Salicylate, as described above, Trimethaphan Camphorsulfonate, Trimethadione, Metronidazole or Penicillin O, particularly the water soluble potassium salt.

In the preparation of products of this sort, the emulsion is designed so that the medicinal is only slightly soluble in the external phase so as to provide permeation of the medicinal through the external phase into the GI tract over a period of time. In general, emulsions of this sort are designed so that the medicinal is soluble in the external phase from about 0.0001 wt. % to about 10 wt. % at 37°C.

In carrying out the process of the instant invention, the internal phase is selected according to the above criteria to enable the skilled artisan to carry out the desired operations. For example, when it is desired to provide a composition for the removal of ammonia in the GI tract, an internal phase comprising a 10 normal aqueous hydrochloric acid solution is emulsified in a hydrocarbon

solution containing a nonionic surfactant along with a thickener for the hydrocarbon phase. This thickener, as will be further described below, is utilized to provide emulsions which do not break during passage to the GI tract since it would be quite evident to the skilled artisan that the advantages of the instant invention will not be obtained with emulsions that are not stable during passage through the GI tract. The aqueous and hydrocarbon mixture is emulsified under vigorous agitation to form a stable emulsion. In this procedure, the aqueous phase is added slowly to the hydrocarbon, surfactant and thickener solution over a period of time to form an oil continuous emulsion. This emulsion may be passed directly by ingestion through the GI tract; however, in the preferred embodiment, this emulsion will be mixed under conditions of low agitation with water to provide a three-phase system. This three-phase system may be then ingested and subsequently passed through the GI tract. This particular emulsion will pass through the stomach into the intestines wherein ammonia present therein will permeate through the external phase of the emulsion, i.e., the hydrocarbon continuous phase, into the aqueous hydrochloric acid phase wherein the ammonia will be converted to ammonium chloride which is impermeable and thus trapped in the internal phase. The emulsion, being stable during passage to the GI tract, then will be passed out of the human body carrying the ammonia trapped in the internal phase along with it.

The following are other specific embodiments of the instant invention, however, there is no intention to be bound by these embodiments since variations which would be obvious to the skilled artisan may be made.

EXAMPLE 2 - Ammonia Removal

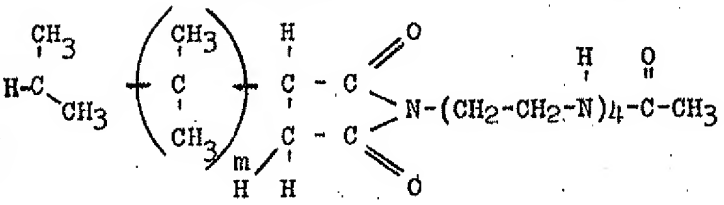
Liquid membrane encapsulation, that is, utilizing the exterior phase of an emulsion as a membrane allows one to use effective ionic reagents such as hydrochloric acid, which cannot be used with other encapsulation methods. A hydrocarbon base liquid membrane is used. The ionic barrier character of this membrane prevents the hydrogen and chlorine ions of this totally ionized strong acid from penetrating the membrane to the bulk fluid (which would be the gut fluid in this application). The species to be removed, ammonia, always exists in equilibrium with the ammonium ion ($NH_3 + H^+ \rightleftharpoons NH_4^+$). Which form is dominant depends on the pH. Ammonia, the molecular species NH_3, which exists at gut pH's, can readily penetrate the liquid membrane to contact the hydrochloric acid reagent. At the very low pH of the encapsulated hydrochloric acid, the molecular ammonia which has moved through the liquid membrane is converted to ammonium (NH_4^+). This ionic species is prevented from transferring back out by the ion barrier properties of this liquid membrane.

The liquid membrane encapsulated hydrochloric acid is shown to be effective experimentally. For any reagent system to be effective in the gut, it must remove ammonia from the very low concentrations which are found in the gut. Tests with liquid membrane encapsulated hydrochloric acid reduced the ammonia concentration of a solution down to less than 3 mg %, i.e. 3 mg per 100 cc's.

In addition to removing ammonia to low levels, small reagent volumes are highly desirable. This could be accomplished by using liquid membrane encapsulated concentrated, 10 normal, hydrochloric acid. In this experiment, the liquid membrane oil phase was made from:

2 gm of Sorbitan monooleate

0.5 gm of a high molecular weight polyamine with the structure



4.5 gm of a polyisobutylene with an average molecular weight of about 900.

93.0 gm of an isoparaffinic lubricating oil stock with a viscosity at 100°F of about 600 Saybolt Universal seconds

100 gm of oil phase, total

To the above 100 gms. of oil phase, 50 gm of 10N hydrochloric acid was added in a progression of drops with vigorous agitation to form an emulsion. One gram of this emulsion was added to 100 gms of dilute ammonia solution in a beaker. The combination was stirred with a propeller at a very slow 50 rpm to give very mild agitation. This agitation is probably milder than naturally occurs in the gut. As too mild an agitation can produce slow removal, it was a severe test. The very encouraging rapid removal of ammonia obtained is shown below in Table 3.

Table 3

Ammonia Removal by Liquid Membranes

Contact Time (Hours)	Ammonia Concn. in Bulk Phase (mg%)
0	26
1/2	21
2	10
24	6

Note that the ammonia level was reduced from 26 mg % to 10 mg % in the first two hours of this gentle contacting.

The level dropped to 6 mg % in 24 hours. The effectiveness of ammonia removal was also quite encouraging. Based on the ammonia removal achieved in this experiment with 1 gm of liquid membrane encapsulated reagent, the quantity required to remove all of the nitrogen from 12 gm/day of urea was calculated. Only 300 cc of emulsion per day is required. The use of a liquid membrane suspension, i.e. the above described emulsion suspended in an aqueous phase, wherein 40 volume percent of the emulsion was concentrated hydrochloric acid, would lower the requirements to 100 cc's for removal of all the urea nitrogen.

The liquid membrane must also function in gut fluid. To test this, a synthetic gut fluid was prepared. The synthetic gut fluid was made with 0.5 wt. % NaCl to simulate salt concentration, buffered with 0.4 wt. % $NaHCO_3$ to hold the proper pH and contained 0.8 wt % egg albumin to simulate protein content. The same type of experiments described above were performed. The results, below in Table 4, show quite clearly that the liquid membrane encapsulated hydrochloric acid removes ammonia from synthetic gut fluid.

Table 4

Ammonia Removal From Synthetic Gut Fluid

Contact Time (Hours)	Ammonia Concn. in Synthetic Gut Fluid (mg %)
0	38
1	19
24	20
48	13

Another quite interesting observation was made when contacting the above-described emulsion with synthetic gut fluid. The stability was enhanced. This may

be a result of protein adsorption on the suspended emulsion droplets. The enhanced stability in gut fluid may play an important role in the *in vivo* emulsion stability discussed below.

The hydrochloric acid reagent could be replaced with citric acid, or any other acid capable of neutralizing ammonia, in the above example.

EXAMPLE 3 - Urease Encapsulation

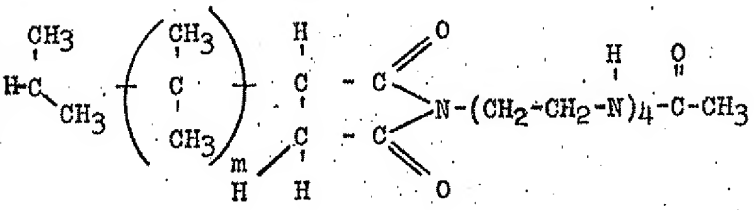
The approach discussed above concerned the removal of ammonia which had been generated from urea by the enzyme urease. Substantial urease activity in the gut has been established by the literature. However, it has not been conclusively proved that there is sufficient urease activity to convert all the urea that must be removed each day. It might be necessary to introduce more urease activity to the gut. Simple injection of unencapsulated urease would not be likely to be effective as the low pH of the stomach would denature much of the enzyme. Therefore the encapsulation of urease was tested in a neutral solution by an ion excluding liquid membrane. The ion exclusion nature of the liquid membrane would prevent the hydrogen ions present at the low pH of the stomach from penetrating the membrane and damaging the urease. The molecular species, urea, however, could readily transfer through the membrane where it would be hydrolyzed to ammonia and carbon dioxide. The carbon dioxide, again a molecular species, could transfer back out through the membrane. The 9 grams per day of carbon dioxide produced from 12 gms per day of urea could readily be handled by the lungs. The ammonia produced by the urease encapsulated in the liquid membrane could transfer out through the liquid membrane. This occurs because the phase encapsulated in these membranes is

near neutral. At near neutral pH's the main species is un-ionized ammonia which can transfer out of the ion barrier liquid membrane. The ammonia leaving the encapsulated urease may then be removed from the gut fluid by the previously discussed ammonia trapping.

The system described above was experimentally checked for the transfer of reactant and products into and out of the urease containing internal phase as well as the activity and effective isolation of the urease. A liquid membrane forming emulsion was made by dissolving 0.046 wt. % urease in water and adding it dropwise into an oil phase under vigorous agitation. The oil phase consisted of:

2 wt. % Sorbitan monooleate

3 wt. % High molecular weight polyamine with the structure



95 wt. % Isoparaffinic lubricating oil stock with a viscosity at 100°F of about 100 Saybolt Universal Seconds

In the final emulsion, the weight ratio of the urease solution to oil phase was 0.82. Two ml of the above emulsion was added to 30 ml of a solution containing 0.43 Molar Urea, 0.1 Molar NaCl, 0.0008 Molar phosphate buffer and containing 0.1μ of Clelands reagent. Moderate stirring was used to disperse the emulsion in liquid membrane form. The pH of this bulk urea containing solution was held at 6.7 $\pm$ 0.05 by an automatic titrator which neutralized the excess product ammonia with 10 normal HCl. (At the 6.7 pH one-half of the ammonia produced is in excess over the quantity forming bicarbonate with the carbon

dioxide.) In these experiments, the quantity of HCl required to balance the excess product ammonia was recorded with time. The liquid membrances were removed during the experiments and reintroduced at a later time.

Increasing the HCl was required initially, indicating that the enzyme catalyzed reaction as well as the transfer of urea into and carbon dioxide and ammonia out of the urease containing internal phase was occurring. When the emulsions were removed, the reaction stopped. This shows that the enzyme did not penetrate the liquid membrane to the bulk phase and that the initial measured reaction rate was that produced by liquid membrane encapsulated urease. Reintroduction of the emulsion started the reaction again. The formation of ammonia in these experiments was also confirmed by independent specific analysis of ammonia built up with time.

The rates of reaction were about 1/50 of those measured under similar conditions with freshly dissolved urease in the unprotected bulk phase. This is a reasonable rate and the reduction from bulk phase includes the effects of several factors. The denaturation of the enzyme during encapsulation, and any limitations in transferring material through the liquid membrane or inside the encapsulated phase would all decrease the measured urease activity.

EXAMPLE 4- Phosphate Removal

Since the phosphate ion is difficult to remove by hemodialysis an adjunct method of removal would be particularly useful. The reagent system selected for encapsulation is suggested by nature. Excess phosphate in the body can precipitate with calcium in non-physiologic

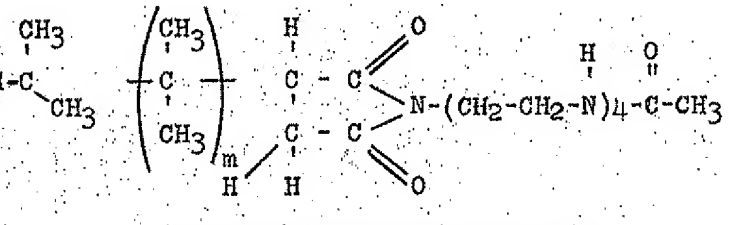
modes. The system selected encapsulates calcium salts in an anion transferring liquid membrane. The cation calcium is retained in the liquid membrane. The anion phosphate transfers through the liquid membrane to react with the calcium forming the calcium phosphate precipitate which is trapped in the internal emulsion phase.

This system was experimentally tested using a 15 weight percent $CaCl_2$ and a 5 weight percent $Ca(OH)_2$ reagent encapsulated in an anion transporting liquid membrane. The oil phase of this emulsion consisted of

95 wt. % Isoparaffin lubricating oil stock with a viscosity at 100°F of about 100 Saybolt Universal Seconds

2 wt. % Mixture of primary and secondary amines with a molecular weight range of 353 to 393 which has an ion exchange capacity of about 2.7 meg/gm., e.g. Amberlite LA 2 available from Rohm and Haas

2 wt. % Polyamine with a molecular weight of about 2000 with the structure



1 wt. % Sorbitan monooleate

The aqueous phase was added to the oil to form 33 wt. % of the total aqueous plus oil phase with vigorous agitation. This emulsion (281 gms) was then dispersed in a phosphate solution (500 gm). The rapid phosphate removal is shown in Table 5 below.

Table 5

Rapid Phosphate Removal

Time (min)	Phosphate (wt. %)
0	0.273
2	0.123
5	0.073
18	0.016
44	0.004

Assuming the removal of all the phosphate ion (1/2 gms/day as phosphorous) was desired, the quantity of liquid membrane suspension, i.e. the emulsion suspended in an aqueous phase, required can be calculated. Based on the above reagent concentration and the reagent occupying 40 volume percent of a liquid membrane suspension, 57 cc would be required per day.

Example 5 - In Vivo Stability of Emulsions

Emulsions that are used to treat chronic uremia by ingestion must be stable throughout the gastrointestinal tract. As a critical test of stability, high doses of a poison were encapsulated in an emulsion to see if the stability of the liquid membrane barrier was sufficient to prevent killing test animals. The poison selected was sodium cyanide at 10 times the lethal dose (10 x LD 50). The liquid membrane formulation was the same ion excluding formulation which was used in removing ammonia from solution and synthetic gut fluid. Wistar-strain, albino rats were used for this study. In addition to the rats used to determine the LD 50 of this population, three groups of 10 rats were used. One group received distilled water encapsulated in the liquid membrane. A second group received 10 times the lethal dose of sodium cyanide encapsulated in the liquid membrane. The encapsulated aqueous phase was 0.5 wt. % sodium cyanide. This sodium cyanide

solution was emulsified at a 33 wt. % level in the same oil phase composition as usual in the ammonia removal examples. This emulsion was then suspended in an equal volume of water prior to administration. In the third group, the hydrocarbon solution and the sodium cyanide solution were introduced as separate liquids so there were no liquid membranes. All the materials were administered by oral intubation. The results are summarized below in Table 6.

TABLE 6

LIQUID MEMBRANE STABILITY IN VIVO

Time After Administration	Group 1 Liquid Membrane Encapsulated H_2O	Group 2 Liquid Membrane Encapsulated HCN	Group 3 Same as Group 2 Not Encapsulated
5 min.	active, feeding	active, feeding	all knocked down
30 min.	active, feeding	active, feeding	all dead
1 hr.	active, feeding	active, feeding	
2 hr.	active, feeding	active, feeding	
1 day	active, feeding	active, feeding	
7 days	active, feeding	active, feeding	

Additionally, the rats administered 10 times the lethal dosage of NaCN in the emulsion were observed to have no signs of toxicity or pharmocologic effects throughout the test. It was concluded that the emulsions of the instant invention have good stability in vivo.

SUBSTITUTE

REMPLACEMENT

SECTION is not Present

Cette Section est Absente